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Luteinizing hormone secretion as influenced by age and estradiol in the prepubertal gilt

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ABSTRACT

The aim of this study was to determine if there is an age related reduction in the sensitivity of the negative feedback action of 17β-estradiol (estradiol) on luteinizing hormone (LH) secretion in the prepubertal gilt. Ovariectomized gilts at 90 ($n = 12$), 150 ($n = 11$) or 210 ($n = 12$) days of age received estradiol benzoate (EB) osmotic pump implants 6/group and the remaining animals received vehicle control (C) implants except for 150-day C ($n = 5$) on Day 0. On Day 10 blood samples were collected every 15 min for 8 h and serum LH and estradiol concentrations were measured. Serum estradiol concentrations averaged 5 ± 1, 5 ± 1 and 7 ± 2 pg/ml for the 90-, 150- and 210-day-old gilts implanted with estradiol, respectively, whereas, serum estradiol concentrations was undetectable in C gilts. Mean serum LH concentrations, basal LH concentrations and serum LH pulse amplitude were less in EB-treated gilts at all ages compared to control animals. In contrast, LH pulse frequency initially was less in EB-treated gilts but subsequently increased ($P < 0.04$) with age (from 0.8 ± 0.2 at 90 days to 5.2 ± 0.2/8 h at 210 days), and at 210 days of age the pulse frequency was similar to C gilts. These results demonstrate an age related reduction in the sensitivity to the negative feedback action of estradiol on LH secretion and support the idea that the gilt conforms to the gonadostat hypothesis.

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1. Introduction

The occurrence of puberty in the female is the culmination of a series of events that result in estrus, ovulation and normal corpus luteum function. Dierschke et al. (1974) attempted to define puberty based exclusively on the hormonal interactions preceding the first ovulation. He proposed that, in the Rhesus monkey, puberty can be defined as the time when the hypothalamic-hypophyseal unit first becomes responsive to increasing concentrations of circulating estrogens resulting in a surge of luteinizing hormone (LH) and follicle stimulating hormone (FSH). To that extent Ramirez and McCann (1963) proposed the "gonadostat" hypothesis which has provided the conceptual frame work for numerous studies examining the neuroendocrine mechanisms controlling onset of puberty. The hypothesis contends that mechanisms within the hypothalamus that governs gonadotropin release from the pituitary of prepubertal animals are set such that negative feedback by ovarian estrogen on gonadotropin secretion is high. When the animal matures, the "gonadostat" is reset and the hypothalamus becomes less sensitive to the negative feedback of ovarian estrogen. As a result, gonadotropin secretion increases to adult concentrations resulting in follicular development and ovulation.

During the postnatal period various components of the brain–pituitary–ovarian axis of the pig are functional prior to normal onset of puberty (Kraeling and Barb, 1990). Several studies have characterized pulsatile LH secretion in the pig during pubertal development (Pelletier et al., 1981; Lutz et al., 1984; Diekman et al., 1983; Camous et al., 1985).

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In general, mean serum LH concentrations and frequency of LH pulses increase from 15 days of age to a maximum between 110 and 125 days of age, then decrease until 150 days of age and remain suppressed (juvenile nadir) until the peripubertal period. Lutz et al. (1984) and Pelletier et al. (1981) demonstrated that LH secretion immediately preceding puberty was characterized by an increased frequency of lesser amplitude LH pulses compared to previous ages. In addition, Lutz et al. (1984) showed that LH secretion increased concomitantly with increased estrogen concentrations just prior to puberty. Moreover, Berardinelli et al. (1984) demonstrated a reduction in the negative feedback effect of estradiol on LH secretion in ovariectomized (OVX) prepuberal compared to ovariectomized postpuberal gilts.

If the gonadostat hypothesis is applicable to the gilt as it is to the lamb (Foster, 1994) and heifer (Day et al., 1984), an increase in serum LH concentrations before puberty must be evident, and this increase must occur in the face of constant or increasing serum estrogen concentrations. Therefore, the objective of the present study was to determine if there is an age related reduction in the sensitivity to the negative feedback action of estradiol on LH secretion in the prepubertal gilt.

2. Materials and methods

Crossbred gilts were ovariectomized (OVX) at 90 ($n=12$), 150 ($n=11$) and 210 ($n=12$) days of age and were individually penned in an environmentally controlled building and exposed to a constant temperature of 22 °C and artificial 12:12 h light:dark photoperiod. Pigs were meal-fed daily at 0800 and 1700 h, a corn-soybean meal ration (14% crude protein) supplemented with vitamins and minerals, according to the National Research Council guidelines (NRC, 1998). Body weights were recorded at the start of the study (Day 0). On Day 0, pigs from each age group received an Alzet osmotic pump (model 2ML2; Alza Corp., Palo Alto, CA) subcutaneously (sc) behind the ear, which contained the appropriate amount of estradiol benzoate (EB; Sigma) in polypropylene glycol vehicle to administer 0.19 mg EB per kg body weight per day. The remaining animals received pumps containing vehicle (control; C). Thus, there were six pigs per treatment except for 150-day C ($n=5$). The study was conducted in two replicates with three pigs per treatment/age group in the first replicate. All pigs were fitted with an indwelling jugular vein cannula (Barb et al., 1982) 24 h before blood sample collection. On Day 10 pigs were fed at 08:00 h and blood samples were collected every 15 min for 8 h. Serum was harvested and stored at −20 °C until assayed for LH and 17β-estradiol (estradiol). All procedures were approved by the Richard B. Russell Agriculture Research Center Committee on Animal Care and Use.

2.1. Radioimmunoassay

Serum samples were assayed for LH (Kesner et al., 1987) as previously described. Sensitivity of the assays was 0.15 ng/ml, and intra- and inter-assay coefficients of variation were 4.2% and 10.5% respectively. Estradiol data were previously reported by Qian et al. (1999).

2.2. Statistics

For each gilt, within age and treatment, mean serum LH concentrations, basal serum LH concentrations, number of serum LH pulses and serum LH pulse amplitude were determined by Pulsar analysis, using a 1% criterion of variation (Merriam and Wachter, 1982) during the post-treatment period. Serum LH, E and body weight data were subjected to a split-plot-in-time ANOVA using the general linear model procedures of Statistical Analysis Systems (SAS, 1999). The statistical model included treatment, age, pig, and replicate. Differences between treatments within an age or between ages within a treatment were determined by least-squares contrasts.

3. Results

There was no effect of replicate ($P>0.20$) detected, thus data were combined across replicates. Body weights (kg) increased ($P<0.05$) with age with no difference between C and EB gilts and averaged 44 ± 4 compared to 50 ± 4 kg (90 days), 82 ± 4 compared to 83 ± 4 kg (150 days) and 113 ± 4 compared to 117 ± 4 kg (210 days), respectively. Serum estradiol concentrations (pg/ml) were less ($P<0.01$) in C than in EB pigs and were similar across ages within treatment and averaged 4 ± 1 compared to 10 ± 1 pg/ml (90 days), 2 ± 1 compared to 8 ± 1 pg/ml (150 days) and 4 ± 1 compared to 10 ± 1 pg/ml (210 days), respectively. Mean serum LH concentrations, basal serum LH concentrations and serum LH pulse amplitude were less in EB-treated gilts at all ages compared to control animals (Fig. 1). In contrast, LH pulse frequency initially was lower in EB-treated gilts but subsequently increased with age (from 0.80 ± 0.2 at 90 days to 5.2 ± 0.2/8 h at 210 days) and at 210 days of age the pulse frequency was similar to C treated gilts ($P=0.2$; Fig. 1).

4. Discussion

These results demonstrate that the neuroendocrine axis became less sensitive to the suppressive effects of exogenous estradiol on LH secretion as gilts progressed from the prepubertal to peripubertal state. This maturational change in sensitivity to estrogen may reflect decreased GnRH neuronal estradiol receptor (ER) concentration and (or) a change of ERα or ERβ receptor subtypes, thereby altering neuron activity. The presence of both ERα and ERβ in rat GnRH neurons was identified (Hu et al., 2008) and selective activation of ERβ increased GnRH secretion, while activation of ERα reduced GnRH secretion. This action was dose dependent, and thus, demonstrating dual action of estradiol on GnRH neuronal activity (Hu et al., 2008). It is plausible that a similar mechanism could modulate GnRH neuronal activity in the pig but it should be noted that the presence of ERα or ERβ in pig GnRH neurons has not been demonstrated. However, only ERα appears to be critical for estrogen-negative feedback suppression of GnRH mRNA in mice (Dorling et al., 2003). Whether or not the

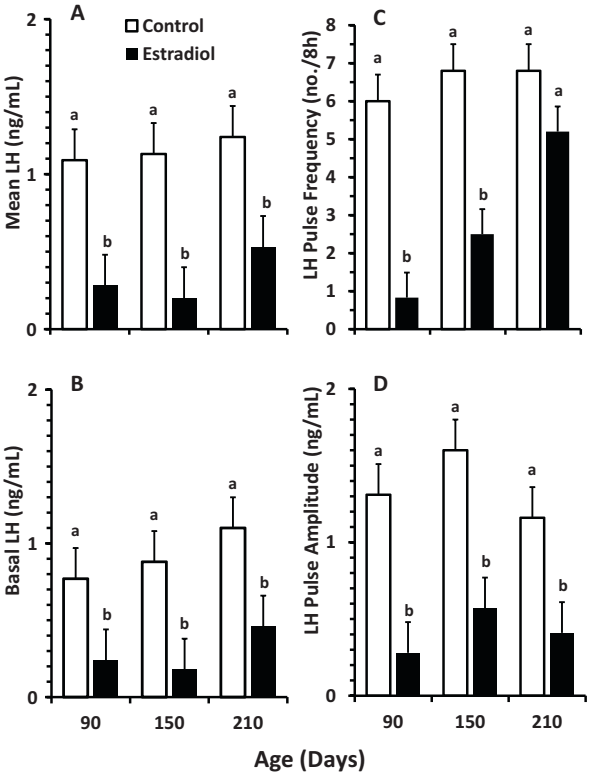

Fig. 1. Serum luteinizing hormone (LH; mean ± SE) concentrations (A), basal serum LH concentrations (B) and frequency (C) and amplitude (D) of serum LH pulses for ovariectomized (OVX) prepuberal gilts at 90, 150, and 210 day of age implanted with osmotic pumps containing estradiol or vehicle control ($n = 6$ per treatment/age except 150 day old control, $n = 5$). Amplitude = pulse height − basal concentration. Blood samples were collected every 15 min for 8 h. [a,b]Columns with different superscripts differ ($P < 0.04$).

pubertal decrease in sensitivity to estrogen negative feedback is a result of related changes in GnRH neuronal steroid hormone receptor gene expression remains unclear.

Alternatively a reduction in inhibitory inputs and (or) an increase in stimulatory inputs to the GnRH neuron (Ojeda and Terasawa, 2002; Sisk and Foster, 2004) and (or) a decline in ER concentrations in intermediary neuronal pathway that regulates GnRH secretion may play a primary role in activating the GnRH neurons (Day et al., 1984; Dierschke et al., 1974). To that extent, Day et al. (1984) observed in the heifer that the number of cytosolic ER declined in the anterior and medial basal hypothalamus and anterior pituitary in association with increased LH secretion and onset of puberty. In the prepubertal pig, concentrations of cytoplasmic and nuclear hypothalamic ERβ were not different at 4 and 5.5 months of age (Diekman and Anderson, 1983). We observed no change in hypothalamic ERβ between 150 and 210 days of age but ERα increased during this time (C.R. Barb, unpublished). Thus, it is reasonable to question whether a developmental change in hypothalamic ER mRNA represents a change in sensitivity to steroid negative feedback or type of ER may be of greater importance as cited above. Further work is needed to substantiate this idea.

Kisspeptin, a peptide product of the KISS1 gene (Ohtaki et al., 2001), is a neuropeptide proposed to regulate reproduction by activating the G-protein coupled receptor, GPR54 (Ohtaki et al., 2001). Genetic knockout mutation that disrupts the kisspeptin/GPR54 signaling results in pubertal failure due to hypogonadotropism (Seminara et al., 2003; de Roux et al., 2003). Kisspeptin stimulates LH and FSH release in the prepubertal gilt (Lents et al., 2008). The kisspeptin gene is expressed in the arcuate (ARC) and periventricular nucleus (PeVN) of the hypothalamus in the pig (Tomikawa et al., 2010). Kisspeptin neural fibers project to GnRH neurons (Pompolo et al., 2006) and the GPR54 gene is expressed in these neurons (Han et al., 2005). Furthermore, repeated intra-cerebroventricular injections of kisspeptin to prepubertal female rats increased LH secretion and advanced the onset of puberty as indicated by vaginal opening (Navarro et al., 2004). Therefore, kisspentin is generally regarded as a primary "gatekeeper" of pubertal activation of the GnRH/LH pulse generator (Seminara et al., 2003; Messager, 2005). A recent report by Tomikawa et al. (2010) demonstrated that upregulation of KISS1 gene expression in the PeVN in mature ovariectomized-EB-treated gilts was sufficient to induce an ovulatory surge of LH. Further study by these authors suggested that the most caudal region of kisspeptin neurons may mediate negative feedback effect of estrogen on GnRH release and subsequent LH secretion, whereas the PeVN kisspeptin neuron most likely has a role in estrogen positive feedback regulation to induce a GnRH/LH pre-ovulatory surge in the pig.

In summary, we have clearly demonstrated an age related reduction in the sensitivity to the negative feedback action of estradiol on pulsatile LH secretion in the prepubertal gilt. Moreover, we suggest that these changes in the gonadostat may be mediated in part by upstream developmental changes in neuronal regulatory control of GnRH secretion such as kisspeptin-GPR54 signaling pathway. Further work is needed to substantiate this hypothesis.

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